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# Dual hypothermic oxygenated machine perfusion in liver transplants donated after circulatory death

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**Background:** Experimental studies have suggested that end-ischaemic dual hypothermic oxygenated machine perfusion (DHOPE) may restore hepatocellular energy status and reduce reperfusion injury in donation after circulatory death (DCD) liver grafts. The aim of this prospective case-control study was to assess the safety and feasibility of DHOPE in DCD liver transplantation.

**Methods:** In consecutive DCD liver transplantations, liver grafts were treated with end-ischaemic DHOPE. Outcome was compared with that in a control group of DCD liver transplantations without DHOPE, matched for donor age, donor warm ischaemia time, and recipient Model for End-stage Liver Disease (MELD) score. All patients were followed for 1 year.

**Results:** Ten transplantations involving liver grafts treated with DHOPE were compared with 20 control procedures. There were no technical problems. All 6-month and 1-year graft and patient survival rates were 100 per cent in the DHOPE group. Six-month graft survival and 1-year graft and patient survival rates in the control group were 80, 67 and 85 per cent respectively. During DHOPE, median (i.q.r.) hepatic adenosine 5'-triphosphate (ATP) content increased 11-fold, from 6 (3–10) to 66 (42–87)  $\mu\text{mol per g protein}$  ( $P = 0.005$ ). All DHOPE-preserved livers showed excellent early function. At 1 week after transplantation peak serum alanine aminotransferase (ALT) and bilirubin levels were twofold lower in the DHOPE group than in the control group (ALT: median 966 versus 1858 units/l respectively,  $P = 0.006$ ; bilirubin: median 1.0 (i.q.r. 0.7–1.4) versus 2.6 (0.9–5.1) mg/dl,  $P = 0.044$ ). None of the ten DHOPE-preserved livers required retransplantation for non-anastomotic biliary stricture, compared with five of 20 in the control group ( $P = 0.140$ ).

**Conclusion:** This clinical study of end-ischaemic DHOPE in DCD liver transplantation suggests that the technique restores hepatic ATP, reduces reperfusion injury, and is safe and feasible. RCTs with larger numbers of patients are warranted to assess the efficacy in reducing post-transplant biliary complications.

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[Correction added on 13th April 2017, after first online publication: 'grafts' removed from the article title]

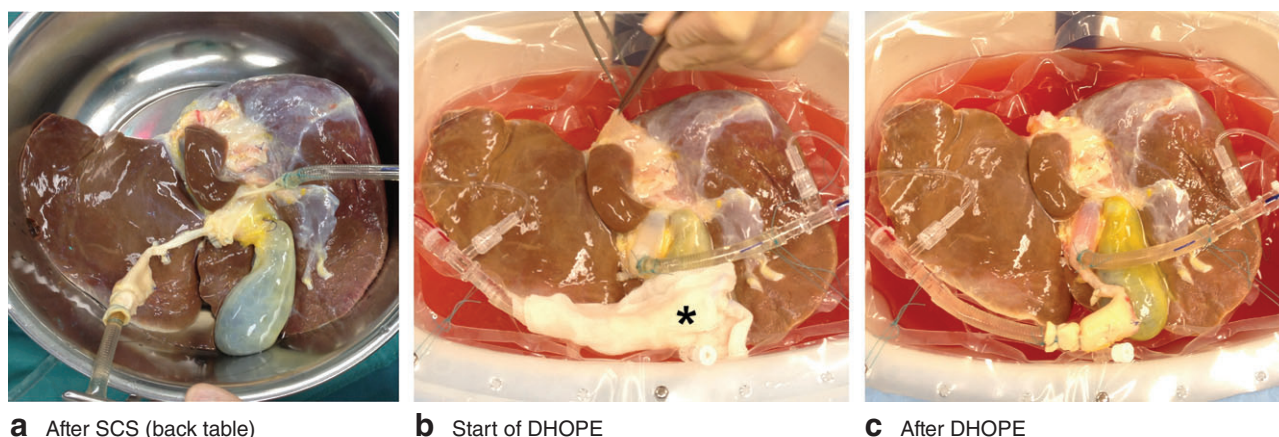
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## Introduction

Donation after circulatory death (DCD) liver grafts are used increasingly for transplantation in an attempt to overcome the discrepancy between the number of available donors and the number of patients waiting for a liver transplant. A major drawback of DCD livers compared

with donation after brain death (DBD) livers is the inevitable period of warm ischaemia between withdrawal of life support and circulatory arrest. This first period of warm ischaemia and the subsequent cold ischaemia during transportation lead to depletion of intracellular energy sources, such as adenosine 5'-triphosphate (ATP), as well as



**Fig. 1 a–c** Macroscopic view of a donor liver during machine perfusion. Liver graft with cannulas in the portal vein and supratruncal aorta during back-table preparation, and before and after dual hypothermic oxygenated machine perfusion (DHOPE). The asterisk indicates a wet sterile gauze protecting the arteries. SCS, static cold storage

other metabolic perturbations causing cellular injury and dysfunction<sup>1,2</sup>. Graft damage is exacerbated by reperfusion injury, and manifests clinically as an increased risk of complications and graft failure after transplantation<sup>3</sup>. The most frequent complications after DCD liver transplantation are biliary complications<sup>4,5</sup>, which include a spectrum of cholangiopathies causing cholestasis, jaundice and cholangitis that may lead to graft loss. Non-anastomotic biliary stricture (NAS) has been reported in up to 30 per cent of patients following DCD liver transplantation – almost three times higher than in recipients of a DBD liver<sup>3</sup>.

Experimental studies<sup>6–13</sup> have suggested that machine preservation may provide better protection of liver grafts against ischaemia–reperfusion injury than the traditional method of static cold storage (SCS). Guarrera and colleagues<sup>14,15</sup> were the first to report successful clinical transplantation of extended criteria DBD donor livers after *ex situ* hypothermic machine perfusion (4–6°C) via the portal vein and hepatic artery without active oxygenation. Dutkowski and co-workers subsequently reported the application of hypothermic (10°C) oxygenated perfusion in DCD liver transplantation<sup>16,17</sup>. Although Dutkowski's group applied active oxygenation of the perfusion fluid, they perfused only via the portal vein and not via the hepatic artery. It is well known, however, that blood supply to the bile ducts is largely dependent on the hepatic artery<sup>18</sup>. Preservation of the biliary tree is critical, and single portal perfusion may not be sufficient to protect the bile ducts, especially in DCD liver grafts. Dual hypothermic oxygenated machine perfusion (DHOPE) combines the advantages of the two above-mentioned techniques: active oxygenation and perfusion via both the portal vein and the

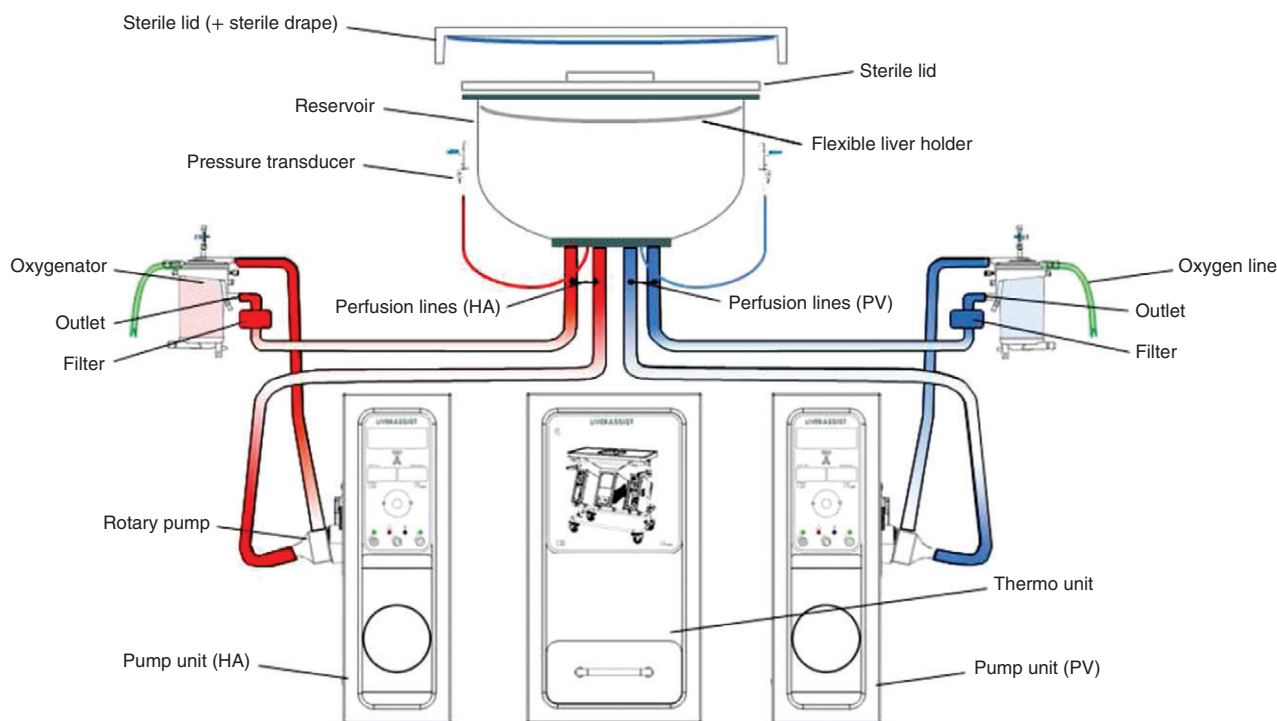
hepatic artery<sup>19</sup>. The hypothesis for the present study was that DHOPE is feasible and safe in resuscitating DCD liver grafts.

## Methods

Between April 2014 and November 2014, consecutive patients (aged at least 18 years) undergoing DCD liver transplantation (Maastricht type 3 and donor bodyweight above 40 kg) with DHOPE at the authors' centre were included in the study. Exclusion criteria for the study were: inability to give informed consent; high urgency status; human immunodeficiency virus positivity; pregnant or nursing; donor positive for hepatitis B or C; or an expected cold ischaemia time greater than 8 h. All livers were allocated according to the regular Eurotransplant rules based on blood type compatibility and Model for End-stage Liver Disease (MELD) score<sup>20</sup>. At the time of listing, patients gave informed consent to the possibility of receiving a DCD graft. At the time of donor liver offer, patients gave informed consent for machine preservation of the donor liver. The study protocol was approved by the institutional medical ethics committee (METc University Medical Centre Groningen; record M14.152454) and was published in an open access registry ([www.trialregister.nl](http://www.trialregister.nl); trial ID NTR4493).

## Donor organ procurement and preparation

After circulatory death of the donor and a 'no-touch' interval of 5 min, aortic flush was performed with at least 4 litres of ice-cold (0–4°C) Belzer University of Wisconsin (UW®) Cold Storage Solution (UW CSS) (Bridge to Life, London, UK) to which 50 000 units of heparin was



**Fig. 2** Schematic drawing of the set-up of dual hypothermic oxygenated machine perfusion. The liver graft was placed in the reservoir, which was covered with a transparent lid to maintain a moist and sterile environment. The system was both pressure and temperature controlled. Two rotary pumps separately provided a pulsatile flow to the hepatic artery (HA) at a mean of 25 mmHg (pressure variation between 20 and 30 mmHg) and a continuous flow to the portal vein (PV) at 5 mmHg. The perfusion fluid was oxygenated by the membrane oxygenators, which also regulated the temperature (set to 10°C). Real-time perfusion flow rates and temperature were measured by sensors and displayed on both pump units

added. Donors were not heparinized systemically before circulatory death. A segment of suprarenal aorta was left attached to the coeliac trunk for later cannulation (Fig. 1). Bile ducts were flushed with preservation solution and a further low-pressure portal flush was performed before packing and transportation in UW CSS. Upon arrival, the livers were flushed through the portal vein with 1 litre of ice-cold Belzer MPS® UW Machine Perfusion Solution (UW MPS) (Bridge to Life).

### Dual hypothermic oxygenated machine perfusion

All livers underwent at least 2 h of DHOPE using the Liver Assist device (Organ Assist, Groningen, The Netherlands). Machine perfusion was performed simultaneously with the recipient hepatectomy. In case of an unexpectedly difficult hepatectomy, DHOPE was prolonged (in 3 instances by 17, 19 and 52 min). The Liver Assist provides pressure-controlled dual perfusion of the liver with rotary pumps (Fig. 2). Arterial pressure was set at 25 mmHg resulting in a pulsatile flow (systolic 30 mmHg, diastolic 20 mmHg) at 60 beats per min. A continuous portal flow

was provided with a pressure of 5 mmHg. Pressure settings were based on previous studies and were subphysiological to avoid shear stress-induced damage of the endothelium at low temperatures<sup>10,19,21</sup>. Four litres of UW MPS, supplemented with 3 mmol/l glutathione, were used as perfusion fluid at a temperature of 10°C. The perfusion fluid was oxygenated by two hollow-fibre membrane oxygenators (100 per cent oxygen at 500 ml/min), resulting in a partial pressure of oxygen of at least 450 mmHg, as described previously<sup>10,19</sup>.

Characteristics of DHOPE such as flow and resistance were assessed every 10 min. Samples of perfusion fluid were collected every 30 min for immediate analysis of perfusate lactate and glucose using an ABL800 FLEX analyser (Radiometer, Brønshøj, Denmark). Additional perfusate samples were centrifuged for 5 min at 2700 r.p.m. at 4°C and stored at -80°C for later biochemical analysis. The concentration of thiobarbituric acid reactive substances (TBARS) was measured in the perfusion fluid as a marker of oxidative stress, as described previously<sup>10</sup>. At the end of DHOPE, a perfusion fluid sample was collected for microbiological testing. Liver wedge biopsies



**Table 1** Donor and recipient characteristics

	DHOPE (n = 10)	Control (n = 20)	P <sup>¶</sup>
<b>Donor characteristics</b>			
Age (years)*	53 (47–57)	53 (47–58)	0.914
Sex ratio (M : F)	5 : 5	13 : 7	0.461#
Body mass index (kg/m <sup>2</sup> )*	23 (20–24)	25 (22–27)	0.044
Cause of death			0.727#
Trauma	4	6	
Postanoxic brain injury	3	5	
Cerebrovascular accident	3	9	
Other	0	0	
Donor highest serum ALT (units/l)*	88 (32–194)	35 (23–99)	0.109
Donor risk index*†	1.89 (1.47–2.19)	2.00 (1.73–2.20)	0.619
Time from circulatory arrest to cold perfusion (min)*	15 (13–17)	16 (14–18)	0.619
Time from withdrawal of life support to cold perfusion (min)*	27 (23–43)	32 (27–39)	0.629
<b>Preservation</b>			
Duration of DHOPE (min)*	126 (123–135)	–	–
Total preservation time (min)*‡	521 (469–592)	503 (476–526)	0.448
<b>Recipient characteristics</b>			
Age (years)*	57 (54–62)	52 (42–60)	0.131
Sex ratio (M : F)	6 : 4	11 : 9	1.000#
MELD score*§	16 (15–22)	22 (17–27)	0.109
Indication for liver transplantation			0.071#
Alcoholic cirrhosis	3	2	
Non-alcoholic steatohepatitis	5	2	
Primary sclerosing cholangitis	1	5	
Primary and secondary biliary cirrhosis	0	2	
Autoimmune hepatitis	0	1	
Hepatitis B or C cirrhosis	1	0	
Hepatocellular carcinoma	0	4	
Cryptogenic cirrhosis	0	3	
Familial amyloid neuropathy	0	1	
<b>Intraoperative characteristics</b>			
Estimated blood loss (litres)*	3.6 (1.8–4.9)	2.6 (2.1–6.6)	0.914
Transfusion of red blood cells (units)*	3 (1.5–7.5)	3 (0.3–7.5)	0.880
Transfusion of FFP (units)*	0 (0–5.5)	0 (0–7.0)	0.914

\*Values are median (i.q.r.). †A validated tool for assessing the risk of liver graft failure<sup>24</sup>; ‡defined as the interval between commencement of aortic cold flush in the donor and portal reperfusion in the recipient; §defined as the highest laboratory-derived Model for End-stage Liver Disease (MELD) score or the (non)-standard exception MELD score. DHOPE, dual hypothermic oxygenated machine perfusion; ALT, alanine aminotransferase; FFP, fresh frozen plasma. ¶Mann–Whitney *U* test, except # $\chi^2$  or Fisher's exact test.

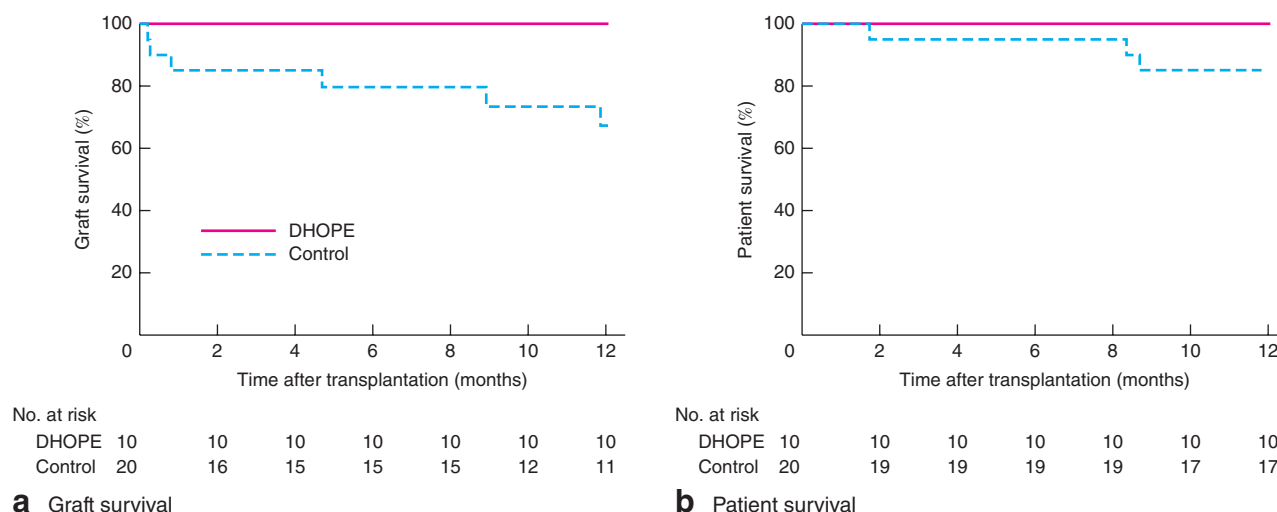
were taken before and after DHOPE, snap-frozen in liquid nitrogen and processed for measurement of ATP concentration<sup>22</sup>.

## Transplantation procedure

Implantations were performed using the piggy-back technique without use of venovenous bypass. Graft reperfusion was initiated via the portal vein with an *in situ* flush of 500 ml of recipient's blood, followed by construction of the arterial anastomosis, using donor common or proper hepatic artery. Biliary reconstruction was performed using duct-to-duct anastomosis without a stent. Immunosuppressive therapy consisted of induction with basiliximab and maintenance immunosuppression with a calcineurin inhibitor (tacrolimus or ciclosporin) and a rapid taper of steroids, either with or without mycophenolate mofetil.

## Control group

Outcome data were compared with those of a matched control group. For each recipient of a DHOPE-preserved graft two control patients were identified within a cohort of patients who underwent primary DCD liver transplantation between 2008 and 2014 at the authors' centre. DCD liver transplantation was initiated at this centre in 2001, and both procurement and implantation techniques have been standardized in a national protocol, which has not been changed over time. The DCD livers of control patients were preserved with conventional SCS only. Matching criteria were based on known risk factors for graft survival: recipient age ( $\pm 5$  years), donor warm ischaemia time ( $\pm 5$  min) and MELD score (less than 22 or at least 23). Donor warm ischaemia time was defined as the time interval between withdrawal of donor life support and initiation of aortic cold flush.



**Fig. 3** Kaplan–Meier curves of **a** graft and **b** patient survival rates within the first year after transplantation in dual hypothermic oxygenated machine perfusion (DHOPE) and control groups. **a**  $P = 0.052$ , **b**  $P = 0.209$  (log rank test)

### Post-transplant outcome

The primary endpoint was graft survival at 6 months after transplantation. Graft survival was defined as the time interval between transplantation and retransplantation or death from graft failure. Secondary endpoints were graft and patient survival rates at 1 year, technical safety of machine perfusion, microbiological testing of perfusion fluid and postoperative complications. Initial poor function was defined based on a modification of the Olthoff criteria: international normalized ratio above 1.6 and/or a serum total bilirubin level greater than 10 mg/dl on postoperative day 7<sup>23</sup>. Serum markers of hepatobiliary injury and function (serum lactate, alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl transferase (GGT), prothrombin time and total bilirubin) were measured using standard biochemical assays.

Other postoperative parameters assessed were duration of ICU and hospital stay, and postoperative complications, including biliary complications such as ischaemic cholangiopathy, defined as NAS or biliary necrosis. NAS was defined as bile duct stenosis at any location in the biliary tree (intrahepatic or extrahepatic, but not at the site of anastomosis) as detected by endoscopic retrograde or magnetic resonance (MRCP) cholangiopancreatography, with clinical signs of cholestasis and/or cholangitis (including raised cholestatic laboratory test results such as for serum GGT and ALP) in the presence of a patent hepatic artery.

Biliary necrosis was defined as evidence of intrahepatic biloma formation or bile duct leakage. All recipients of a DHOPE-preserved liver underwent MRCP 6 months after transplantation. All MRCPs were performed in a routine

manner and assessed by an experienced radiologist, who was not aware of whether a liver had undergone machine perfusion or not.

### Statistical analysis

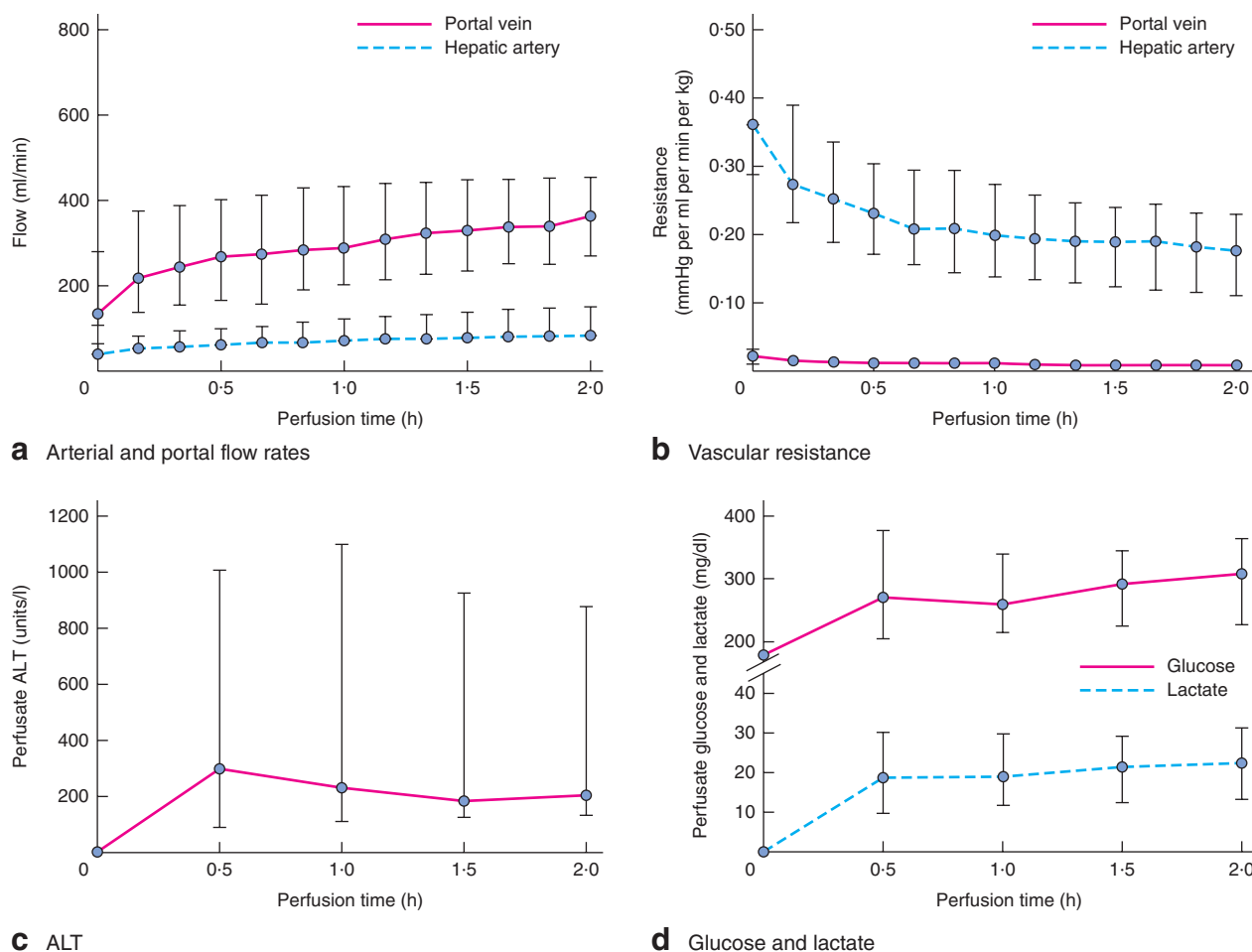
Continuous variables are presented as median (i.q.r.) and compared between groups using the Mann–Whitney  $U$  test. Categorical variables are presented as number and percentage, and compared with the Pearson  $\chi^2$  test or Fisher's exact test. Graft and recipient survival were determined with the Kaplan–Meier method, and significance of survival differences was determined with the log rank test.  $P < 0.050$  was considered to indicate statistical significance. All statistical analyses were performed using SPSS® software version 22.0 for Windows® (IBM, Armonk, New York, USA).

### Results

Ten consecutive, unselected DCD liver transplants had DHOPE preservation before implantation, and no patient had to be excluded based on the set exclusion criteria. Twenty control patients were identified from a cohort of 64. Donor and recipient characteristics of patients in the DHOPE and control groups are summarized in *Table 1*. There were no significant differences in baseline characteristics, except for a lower BMI in the DHOPE group.

### Graft and patient survival

Six-month and 1-year graft and patient survival rates were 100 per cent (10 of 10) in the DHOPE group, whereas



**Fig. 4** Characteristics of dual hypothermic oxygenated machine perfusion. **a** Arterial and portal flow rates were measured by flow sensors attached to the tubing of the perfusion device. **b** Perfusion pressure (mmHg) was measured by pressure sensors attached to the arterial and venous tubing. Vascular resistance was calculated using Ohm's law and expressed as mmHg per ml per min per kg liver. **c,d** Levels of alanine aminotransferase (ALT), glucose and lactate were measured in perfusion fluid samples taken every 30 min during perfusion. Values are median (i.q.r.)

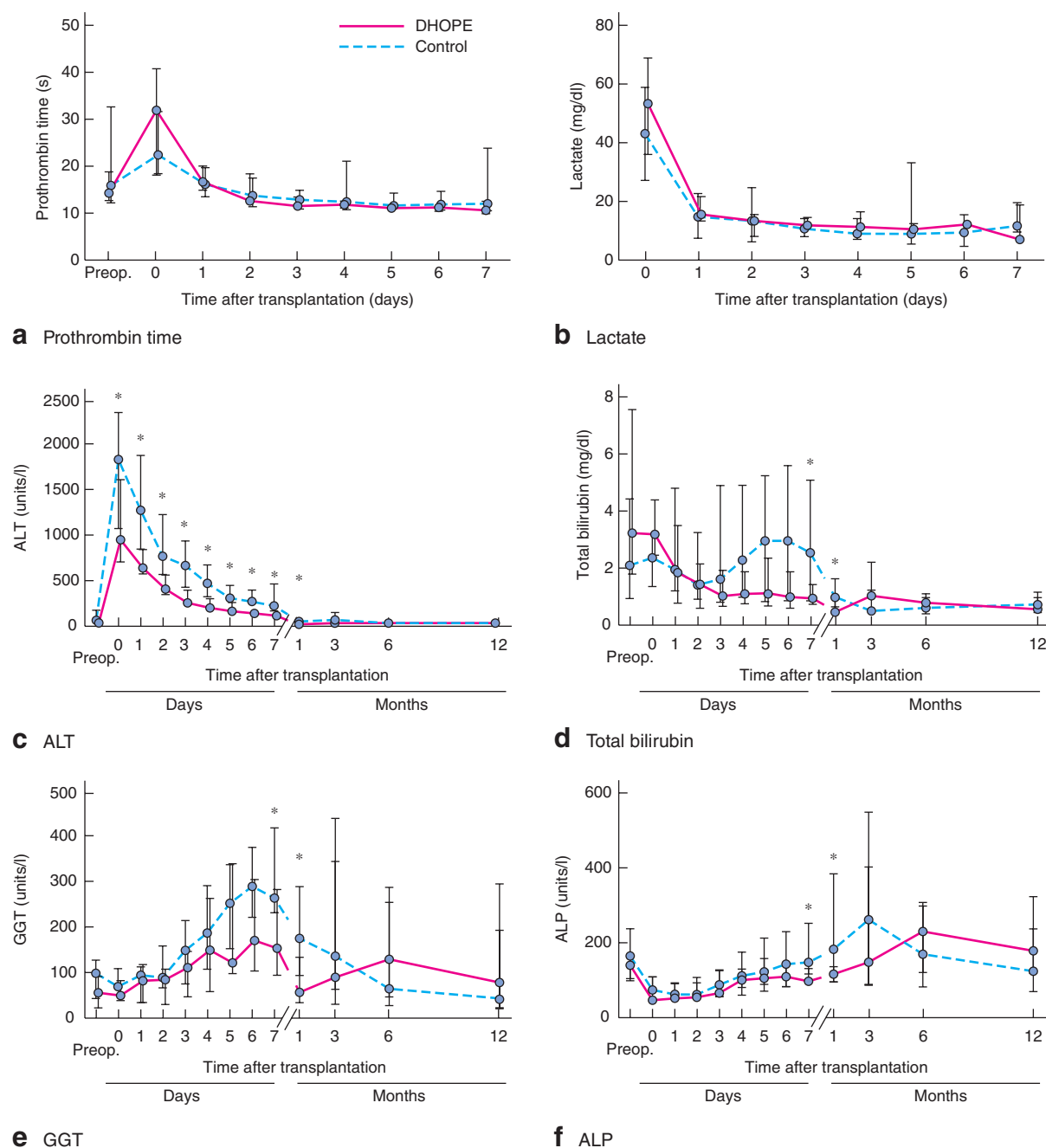
6-month graft survival and 1-year graft and patient survival rates for the 20 patients in the control group were 80, 67 and 85 per cent respectively ( $P=0.052$  for graft survival,  $P=0.209$  for patient survival) (Fig. 3). Graft loss in the control group was due to hepatic artery thrombosis (1 patient), necrotic bile ducts (2) and NAS (3). Patient death in the control group was due to angiosarcoma (1 patient), pneumonia as a complication of treatment for haemophagocytic syndrome (1) and haemorrhagic shock due to intrathoracic bleeding after thoracentesis for pleural effusion (1).

### Characteristics of dual hypothermic oxygenated machine perfusion

No technical problems or device malfunction occurred during machine perfusion. Microbiological evaluation of

the perfusion fluid revealed no evidence of microbial contamination. Livers in the DHOPE group were perfused for a median duration of 126 (123–135) min after a median cold ischaemia time of 331 (308–376) min. This resulted in a total preservation time of 8.7 (7.8–9.9) h for DHOPE *versus* 8.4 (7.9–8.8) h in the control group ( $P=0.448$ ).

The macroscopic appearance of a representative liver graft before and after DHOPE is shown in Fig. 1. Flows increased mainly during the first 30 min of DHOPE and reached a median portal flow of 365 ml/min and a median hepatic arterial flow of 84 ml/min after 2 h (Fig. 4a). In parallel, the vascular resistance decreased during the first 30 min and stabilized thereafter (Fig. 4b). ALT concentration in perfusion fluid increased during the first 30 min of



**Fig. 5** Post-transplant biochemical markers of hepatic injury and function in dual hypothermic oxygenated machine perfusion (DHOPE) and control groups: **a** prothrombin time, **b** lactate, **c** alanine aminotransferase (ALT), **d** total bilirubin, **e**  $\gamma$ -glutamyl transferase (GGT), **f** alkaline phosphatase (ALP). Day 0 was determined as the time interval between reperfusion and midnight. Values are median (i.q.r.). \* $P < 0.050$  (Mann–Whitney  $U$  test)

machine perfusion and decreased thereafter, resulting in a median ALT concentration of 207 (134–878) units/l at the end of DHOPE (Fig. 4c). Lactate and glucose concentrations in the perfusion fluid also increased during the first 30 min and stabilized thereafter (Fig. 4d). There was

no significant increase in the concentration of TBARS in the perfusion fluid during DHOPE (data not shown).

Hepatic ATP concentration increased significantly during DHOPE, from a median of 6 (3–10) to 66 (42–87)  $\mu\text{mol/g}$  ( $P = 0.005$ ). After graft reperfusion in the recipient,



**Table 2** Post-transplant outcomes

	DHOPE (n = 10)	Control (n = 20)	P <sup>†‡</sup>
<b>Recovery</b>			
Peak serum creatinine at ≤ 1 week (mg/day) <sup>††</sup>	1.4 (1.0–2.8)	1.3 (0.8–1.8)	0.373§§
Duration of ICU stay (days) <sup>*</sup>	2 (2–6)	2 (1–5)	0.475§§
Duration of hospital stay (days) <sup>*</sup>	22 (16–33)	23 (15–32)	0.880§§
<b>Complications</b>			
Hypokalaemia (< 3.5 mEq/l) after reperfusion <sup>‡</sup>	3	0	0.030
Initial poor function <sup>§</sup>	0	2	1.000
Primary non-function <sup>¶</sup>	0	0	–
Relaparotomy <sup>#</sup>	3	7	1.000
Renal failure requiring haemodialysis	1	2	1.000
Hepatic artery thrombosis	0	2	0.540
<b>Biliary complications</b>			
Anastomotic biliary stricture	2 <sup>**</sup>	3 <sup>††</sup>	1.000
Biliary cast formation	3 <sup>**</sup>	3 <sup>††</sup>	0.372
Ischaemic cholangiopathy	1	9	0.101
Non-anastomotic biliary stricture	1 <sup>**</sup>	7 <sup>††</sup>	0.210
Massive biliary necrosis	0	2	1.000
Retransplantation for biliary complications	0	5	0.140

\*Values are median (i.q.r.). <sup>†</sup>SI conversion factor: to convert creatinine to micromoles per litre (μmol/l), multiply by 88.4. <sup>‡</sup>SI conversion factor: to convert potassium to millimoles per litre (mmol/l), multiply by 1. <sup>§</sup>Defined based on a modification of the Olthoff criteria: international normalized ratio above 1.6 and/or serum total bilirubin level greater than 10 mg/dl on postoperative day 7<sup>23</sup>. <sup>¶</sup>Determined as retransplantation or death within 7 days of transplantation. <sup>#</sup>Indications for relaparotomy in dual hypothermic oxygenated machine perfusion (DHOPE) group: intra-abdominal blood loss due to diffuse oozing (1); removal of surgical gauzes used for packing to control diffuse oozing during transplantation (1); and biliary anastomotic leakage (1). Indications for relaparotomy in control group: intra-abdominal blood loss due to diffuse oozing (1); removal of surgical gauzes used for packing to control diffuse oozing during transplantation (4); and biliary anastomotic leakage (2). <sup>\*\*</sup>One patient had a combination of anastomotic biliary stricture and biliary cast formation; one patient had biliary cast formation as well as non-anastomotic biliary stricture. <sup>††</sup>One patient had non-anastomotic biliary stricture and later also developed an anastomotic biliary stricture; one patient had biliary cast formation as well as non-anastomotic biliary stricture; two patients had a combination of non-anastomotic biliary stricture and biliary cast formation. <sup>‡‡</sup>χ<sup>2</sup> or Fisher's exact test, except §§Mann–Whitney U test.

hepatic ATP levels were comparable to those at the end of DHOPE (Fig. S1, supporting information).

### Post-transplant hepatobiliary injury and function

Postoperative prothrombin time and serum lactate concentrations were comparable in the two groups during the first 7 days after surgery (Fig. 5a,b). Peak serum ALT levels were significantly lower in recipients of DHOPE-preserved livers compared with levels in controls (median ALT 966 versus 1858 units/l respectively;  $P=0.006$ ) (Fig. 5c). In addition, serum bilirubin concentrations were significantly lower on postoperative day 7 in the DHOPE group: 1.0 (0.7–1.4) mg/dl versus 2.6 (0.9–5.1) mg/dl in controls ( $P=0.044$ ) (Fig. 5d).

Median serum levels of ALT, GGT, ALP and bilirubin at 30 days after transplantation were lower in the recipients of DHOPE-preserved livers than in the control group (ALT: 17 versus 51 units/l respectively,  $P=0.015$ ; GGT: 74 versus 176 units/l,  $P=0.049$ ; ALP: 115 versus 182 units/l,  $P=0.019$ ; bilirubin: 0.5 versus 1.0 mg/dl,  $P=0.019$ ) (Fig. 5c–f). These differences remained significant for ALT and ALP even when patients with NAS on MRCP were excluded from the analyses, suggesting that

DHOPE-preserved livers had less subclinical biliary injury that was not detected by MRCP, compared with controls.

### Post-transplantation outcome

There were no significant differences in kidney function, length of ICU or hospital stay, or incidence of postoperative complications, except for postreperfusion hypokalaemia, which developed in three recipients of a DHOPE liver (Table 2). One recipient of a DHOPE-preserved liver developed NAS in segments II and III of the liver; this was treated successfully with endoscopic stenting. In contrast, seven of 20 patients in the matched control group developed NAS; this was treated successfully with endoscopic stenting in four patients, but required retransplantation in three patients. Both patients in the control group with massive biliary necrosis were retransplanted.

### Discussion

This clinical series of end-ischaemic DHOPE in DCD liver transplantation suggests that this method of liver machine preservation is safe and feasible. Compared with

SCS alone, DHOPE seems to provide better preservation of DCD liver grafts, resulting in a reduction of ischaemia–reperfusion injury and improved early graft function. Graft and patient survival rates after transplantation of DHOPE-preserved DCD livers were 100 per cent during a 12-month follow-up. One-year graft and patient survival rates in the matched controls were 67 and 85 per cent respectively. Although patient survival in the control group was affected mainly by deaths that were not related to graft function or biliary complications, survival rates are consistent with those in recent publications<sup>4,25</sup>.

Hypothermic machine perfusion is a rapidly developing and dynamic field with many still unanswered questions. For example, there is no consensus on the need for active oxygenation. However, experimental data indicate that one of the main benefits of a short period of end-ischaemic oxygenated hypothermic perfusion of DCD livers is the restoration of intrahepatic energy sources. As a result of the periods of warm and cold ischaemia, DCD livers become severely ATP depleted. In the present study, intrahepatic ATP levels increased 11-fold during DHOPE. This restoration of ATP levels during DHOPE is remarkable and can be explained only by an effective extraction and utilization of oxygen from the oxygenated machine preservation fluid by the mitochondria, despite low temperatures. However, because of the low temperatures, the subsequent turnover rate of ATP into adenosine 5'-diphosphate by hepatocytes is low, leading to an accumulation of cellular ATP. This finding is very much in line with data obtained by Dutkowski's group<sup>13</sup> in animal experiments, and by the present authors' group in experimental studies using discarded human donor livers<sup>19,26</sup>. Restoration of ATP levels reduces the cellular 'oxygen debt', resulting in reduced production of radical oxygen species and damage-associated molecular pattern molecules after warm reperfusion in the recipient<sup>6–8</sup>. The downstream effects of this are reduced activation of Kupffer cells and endothelium, limiting ischaemia–reperfusion injury and resulting in a downregulation of the immune response after transplantation<sup>11–13</sup>. Altogether, these data indicate that active oxygenation of the perfusion fluid adds significantly to the benefits of hypothermic machine perfusion.

Another unanswered question is the need for dual *versus* single perfusion of livers. It remains unclear whether dual or single perfusion is equally effective, or whether one method is superior to the other. A potential risk of combined portal and arterial perfusion is mechanical damage that may occur to the hepatic artery, and could cause a higher incidence of hepatic artery thrombosis following transplantation. For this reason, Dutkowski and colleagues<sup>16,17</sup> used only portal vein perfusion. However,

biliary complications are the main obstacle for wider utilization of DCD livers and, based on the dominant arterial vasculature of the biliary tree, single-portal perfusion may not provide optimal preservation of the bile ducts and their vasculature<sup>18</sup>. The present authors have avoided manipulation of the hepatic artery by leaving a segment of supra-truncal aorta attached the donor liver. After machine perfusion, this part of the arterial vasculature was cut off and the donor common or proper hepatic artery was used for anastomosis. None of the patients developed hepatic artery thrombosis. Guarrera and co-workers<sup>14,15</sup> also used combined portal and arterial perfusion without an increased rate of arterial complications.

After transplantation, peak serum ALT levels at 1 week were significantly lower in recipients of a DHOPE-preserved DCD liver than in controls. A high peak ALT following DCD liver transplantation has been identified previously as an independent risk factor for the development of NAS<sup>27</sup>. Only one patient in the DHOPE group developed local NAS, limited to the left lateral segments of the liver, which was treated successfully with endoscopic stenting. The low incidence of NAS is remarkable, especially considering the rather long donor warm ischaemia time (median 27 min), reflecting current DCD practice in the Netherlands<sup>3,25</sup>. The long donor warm ischaemia time probably contributes to the high incidence of NAS of 24–35 per cent in the Netherlands<sup>3,25</sup>. A similar high incidence of ischaemic cholangiopathy was noted in nine of the 20 matched controls, necessitating retransplantation in five patients. A potentially beneficial effect of hypothermic oxygenated machine perfusion on the occurrence of biliary complications after DCD liver transplantation has also been reported by Dutkowski *et al.*<sup>16,17</sup>. However, when donor warm ischaemia time is limited, the benefit of hypothermic machine perfusion may be lower, as short donor warm ischaemia time has been associated with a low risk of NAS and graft failure<sup>28,29</sup>. Formal evidence that hypothermic oxygenated machine perfusion reduces the incidence of biliary complications after DCD liver transplantation should come from RCTs. Based on the present favourable results, a multicentre RCT comparing end-ischaemic DHOPE with SCS alone in DCD liver transplantation has been initiated. The primary endpoint of this study will be NAS within 6 months after transplantation (ClinicalTrials.gov NCT02584283).

Several groups<sup>30–32</sup>, including that of the authors, have reported recently on the feasibility and potential benefits of end-ischaemic normothermic machine perfusion of human donor livers. In contrast to hypothermic machine perfusion, normothermic liver perfusion enables an *ex situ* functional assessment. This facilitates the identification of

transplantable donor livers that would otherwise have been declined because of a high risk of primary non-function. Hypothermic oxygenated machine perfusion does not allow functional assessment of the liver before transplantation, but rather aims to reduce graft dysfunction and complications after transplantation. In this respect, the various types of machine perfusion at different temperatures may prove to be complementary. A major advantage of hypothermic compared with normothermic machine perfusion is its relative simplicity and safety. In addition, hypothermic machine perfusion is associated with lower costs than normothermic machine perfusion.

Limitations of this study are the sample size and use of matched historical controls. DCD liver transplantation was initiated at the authors' centre in 2001, and both procurement and implantation techniques have been standardized in a national protocol, which has not been changed over time. Therefore, no major bias from a learning curve effect when comparing the DHOPE series (performed in 2014) with historical controls performed between 2008 and 2014 would be expected. Primary sclerosing cholangitis (PSC) was the underlying liver disease in five control patients, compared with one patient in the DHOPE group. In general, PSC is not considered a contraindication to DCD liver transplantation as 30 per cent of donor livers come from DCD donors and 15–20 per cent of patients requiring a liver transplant in the Netherlands have PSC as the underlying liver disease. Although patients transplanted for PSC have a higher risk of developing biliary stricture, only one of the five patients with PSC in the control group developed NAS. Therefore, this does not explain the difference in the incidence of NAS between the two groups.

This small clinical study suggests that end-ischaemic portal and arterial hypothermic oxygenated machine perfusion is feasible and safe in resuscitating DCD liver grafts before transplantation. DHOPE resulted in restoration of hepatic ATP content and was associated with a reduction in ischaemia–reperfusion injury, as well as better hepatobiliary function after transplantation. However, the efficacy of DHOPE in reducing (biliary) complications after transplantation remains to be determined in an RCT before the technology is implemented routinely in DCD liver transplantation.

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### Supporting information

Additional supporting information may be found in the online version of this article:

**Fig. S1** Hepatocellular energy levels before and after dual hypothermic oxygenated machine perfusion (DHOPE) in livers in the intervention group only (Word document)